2817-Pos Board B247
Reconstitution and Characterization of Na/K-ATPase in Model Lipid Membranes
Tripta Bhatia.
FKF, Memphys, University of Southern Denmark, Odense M, Denmark.
We have mapped the morphology and orientation of the Na/K ATPase inside model membranes and made a detailed quantifications of the membrane lateral structure. Further work to characterize the lateral organization and function of the pump in membranes and vice-versa, is in progress.

2818-Pos Board B248
Detergent-Free Extraction of the Reaction Center from *Rhodobacter sphaeroides* into Native Nanodiscs. Nanodisc Size Matters!
Stefan Scheidelaar[1], David Swainsbury[2], Martijn Koorengevel[1], Hans Meeldijk[3], Eefjan Breukink[1], Rienk van Grondelle[4], Michael Jones[5], J. Antoinette Killian[1].
[1]Membrane Biochemistry & Biophysics, Utrecht University, Utrecht, Netherlands, [2]School of Biochemistry, Bristol University, Bristol, United Kingdom, [3]Electron Microscopy Utrecht, Utrecht University, Utrecht, Netherlands, [4]Division of Physics and Astronomy, Department of Biophysics, VU University Amsterdam, Amsterdam, Netherlands, [5]School of Biochemistry, Bristol University, Bristol, United Kingdom.
It has been discovered that styrene-maleic acid (SMA) copolymers are able to solubilize membrane proteins directly from their native membrane in the form of nanodiscs [1]. Using the SMA technology, we purified and characterized reaction centers (RCs) from the purple bacterium *Rhodobacter sphaeroides* [2]. Our most significant findings were (i) that the SMA copolymer efficiently solubilizes membranes of this bacterium, (ii) that it allows preservation of the local lipid environment of the solubilized RCs, (iii) that the protein is even more stable in these ''native nanodiscs'' than in the native membrane, and (iv) that the size of the RC containing nanodiscs is significantly larger than that of protein-free nanodiscs.
We next investigated what physical properties of the polymer and lipids determine the efficiency and kinetics of solubilization and how they affect the size of the nanodiscs. In particular, we tested the effect of SMA copolymer length, hydrophobicity, and charge state (varying pH) using different lipid compositions. In complementary assays, we determined how the cross-sectional diameter of a protein affects the size of a nanodisc by using purified constructs of covalently linked oligomeric RCs of different size.
Our study contributes to the fundamental knowledge about the mode of action of SMA and thereby to the general applicability of native nanodiscs as host for membrane proteins and protein complexes of different size.
1. Knowles et al., 2009, JACS, 131, 7484-7485
2. Swainsbury & Scheidelaar et al. 2014, Angewandte Chemie, 53, http://dx.doi.org/10.1002/anie.201406412

2819-Pos Board B249
Membrane-Lipid Mediated Rhodopsin Signaling Involves an Ensemble of Conformational Substates
Udeep Chawla[1], Blake Mertz[2], Eglof Ritter[3], Franz Bartl[4], Michael F. Brown[1,5].
[1]Chemistry and Biochemistry, University of Arizona, Tucson, AZ, USA, [2]Chemistry, West Virginia University, Morangtown, WV, USA, [3]Experimentelle Biophysik, Humboldt-Universität zu Berlin, Berlin, Germany, [4]Charité-Universitätsmedizin, Institut für Medizinische Physik und Biophysik, Berlin, Germany, [5]Department of Physics, University of Arizona, Tucson, AZ, USA.
G-protein-coupled receptors (GPCRs) constitute about 50% of the known drug targets. Using rhodopsin as a model GPCR, we study the role of membrane lipids in the rhodopsin activation. Absorption of light by rhodopsin leads to a series of conformational changes that establishes an equilibrium between inactive Meta-I and an ensemble of activated Meta-II states [1,2]. Using UV-visible and FTIR spectroscopy, we show that the distribution of late conformational substates during rhodopsin activation is lipid-mediated as explained by an ensemble activation mechanism (EAM). Lipid bilayer composition (head groups and acyl chain lengths) and membrane protein-lipid interaction govern the EAM through biasing conformational substates. Rhodopsin reconstituted in DOPC backshifts the equilibrium to the Meta-IIa substate, whereas mixed-chain POPC membranes favor the inactive Meta-I state [3]. The wavenumber-dependent analysis of the FTIR-difference spectra yields a distribution of pK_a and alkaline endpoint values, consistent with an ensemble of substates for each lipid bilayer-rhodopsin system. A phenomenological Henderson-Hasselbalch function was fitted to the pH titration curves to derive thermodynamic parameters from the spectral analysis. Our thermodynamic studies show that activation of rhodopsin is accompanied by an entropy gain compensating the unfavorable enthalpy increase, analogous to protein unfolding reactions. The results from the EAM analysis are in agreement with the flexible surface model (FSM), which describes elastic coupling of the membrane lipids to integral membrane proteins through a balance of curvature and hydrophobic forces in lipid-protein interactions [4]. Our study also provides insight into the thermodynamic parameters that govern rhodopsin-like GPCR activation in native membrane lipid environments. [1] A.V. Struts *et al.* (2011) *PNAS* **108**, 8263-8268. [2] A.V. Struts *et al.* (2014) *Meth. Mol. Biol.* (in press). [3] E. Zaitseva *et al.* (2010) *JACS* **132**, 4815-4821. [4] M.F. Brown (2012) *Biochemistry* **51**, 9782-9795.

2820-Pos Board B250
Inside the Cell under Oxidative Stress: Protein Assembly at Mitochondrial Membranes and its Consequences
Martin Lidman, Artur Dingeldein, Ilona Dudka, Farhana Nasrin, **Gerhard Gröbner**.
Chemistry, Biophysical Chemistry, Umeå, Sweden.
Intracellular oxidative stress is one major factor leading to apoptosis accompanied by the permeabilization of the mitochondrial outer membrane; a process causing release of apoptotic factors such as cytochrome c. Upon onset of intracellular stress phospholipids can become oxidized. These oxidized phospholipids (OxPls) can severely alter the properties of these mitochondrial membranes, and can therefore have i) a direct effect on the membrane properties and its perforation, ii) can change aggregation behavior of amyloidogenic proteins in contact with these membranes iii) can have an indirect effect by altering the function of mitochondrial membrane-coupled Bcl-2 proteins (such as the anti-apoptotic Bcl-2 or the apoptotic Bax), with Bcl-2 potentially involved in SOD1 mediated toxicity [A]. We therefore devised a model system that mimics oxidative stress conditions by incorporating oxidized phospholipids (OxPls) into mitochondria-like liposomes, and studied the OxPls' impact on basic lipid membrane systems and subsequently on protein-membrane interplays. In addition we also carried out a lipidomics profiling of cancer tissues overexpressing Bcl-2 protein to provide information about apoptosis-preventing lipid patterns.

2821-Pos Board B251
Elucidation of a Raft-Partitioning Motif in Transmembrane Proteins
Joseph H. Lorent, Blanca B. Diaz-Rohrer, Kevin J. Spring, Ilya Levental.
Integrative Biology and Pharmacology, UT Health Science Center at Houston, Houston, TX, USA.
Eukaryotic plasma membranes (PMs) are believed to possess lateral lipid domains termed lipid rafts, which form functional platforms for membrane sorting and cell signalling. The function of these domains depends on their selective recruitment of specific membrane proteins. However the underlying structural features governing protein partition into lipid rafts remain unknown. In live cells, the nanoscopic size and subsecond lifetime of rafts presents great difficulty for measuring their properties and composition. Intact PMs isolated as Giant Plasma Membrane Vesicles (GPMVs) phase separate into two microscopic, stable lipid domains, with one of these domains possessing greater lipid order, lower diffusivity and enriching for canonical raft components. This microscopic raft phase separation thus presents an optimal system for measuring protein partitioning between coexisting membrane domains. Using GPMVs, we demonstrate that a protein's transmembrane domain (TMD) is a central determinant of raft affinity. A major factor governing raft partition was the length of the TMD, with longer TMDs preferring the thicker raft domains. Further, by systematic mutation of the TMD of LAT (Linker for activation of T-cells), we were able to determine amino acid sequences which are necessary and sufficient for raft partitioning and which highly resemble the α-helix oligomerization motif GxxxG. This resemblance suggested that TMD oligomerization is the structural basis for the discovered raft-partitioning motif. We confirmed this hypothesis by measure sequence-dependent TMD oligomerization by fluorescence lifetime microscopy (FLIM) combined with Förster resonance energy transfer (FRET). Finally, we demonstrate the biological significance of our observations by showing that raft partitioning determines sorting between organellar membranes. For the entire panel of TMD-variants, we observed a strong quantitative relationship between raft association and sorting to the plasma membrane, with non-raft mutants being sorted into the lysosomes for degradation.

2822-Pos Board B252
The Mechanism of the Disintegration of Phospholipid Membranes with Human Monoacylglycerol Lipase (hMGL)
Vitalii I. Silin[1], Ioannis Karageorgos[2], David J. Vanderah[1], Nikolai Zvonok[3], John Marino[2], Alexandros Makriyannis[4].
[1]IBBR_SG, University of Maryland, Rockville, MD, USA, [2]NIST, Gaithersburg, MD, USA, [3]Northeastern University, Boston, MA, USA, [4]Center for Drug Discovery, Northeastern University, Boston, MA, USA.
In human cancer cells, hMGL is the principal regulator of fatty acids, controlling the hydrolysis of cellular lipids and the production of free fatty